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L2: Entry 7 of 17

File: USPT

Jan 27, 1998

DOCUMENT-IDENTIFIER: US 5712142 A

TITLE: Method for increasing thermostability in cellulase enzymes

Brief Summary Text (6):

The cellulase complex produced by this organism is known to contain several different cellulase enzymes with maximal activities at temperatures of 75.degree. C. to 83.degree. C. These cellulases are resistant to inhibition from cellobiose, an end product of the reactions catalyzed by cellulase. Also, the cellulases from *Acidothermus cellulolyticus* are active over a broad pH range centered about pH 6. A high molecular weight cellulase isolated from growth broths of *Acidothermus cellulolyticus* was found to have a molecular weight of approximately 156,600 to 203,400 daltons by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This enzyme is described in U.S. Pat. No. 5,110,735.

Brief Summary Text (7):

A novel cellulase enzyme, known as the E1 endoglucanase, also secreted by *Acidothermus cellulolyticus* into the growth medium, is described in detail in U.S. Pat. No. 5,275,944. In its native form, this endoglucanase demonstrates a temperature optimum of 83.degree. C. and a specific activity of 40 .mu.mole glucose release from carboxymethylcellulose/min/mg protein. This E1 endoglucanase was further identified as having an isoelectric pH of 6.7. It is this E1 endoglucanase which has been modified and made the subject of this patent application. The E1 endoglucanase is a multidomain cellulase having a catalytic domain and a cellulose binding domain connected to the catalytic domain by a linker peptide.

Detailed Description Text (15):

P. pastoris has been shown to be a useful host organism for expression of large quantities of diverse heterologous proteins. *P. pastoris* was used to express large quantities of active full size E1.

Detailed Description Text (39):

Mutagenized DNA was transformed into *E. coli* strain ES1301. Transformants were screened for resistance to ampicillin and sensitivity to tetracycline in order to identify clones carrying the putatively mutagenized E1 gene. Many ampicillin-resistant candidate clones were subsequently screened on plates containing 1 mM 4-methylumbelliferyl-.beta.-D-cellobioside (MUC) to verify expression of active E1. Plasmid DNA was prepared from several clones and employed as templates in dideoxy DNA sequencing reactions using the Sequenase.RTM. kit (U.S. Biochemical, Cleveland, Ohio) to verify the sequence of E1 DNA in the region of the intended mutation. The mutated sequence was detected in every clone which was sequenced. One of these clones was selected and designated pYCC101. Each of the successfully mutated clones expresses a protein not present in control cells and which migrates at a molecular weight of approximately 42 kDa in SDS-PAGE gels. This 42 kDa protein also reacts with a monoclonal antibody specific for the E1 endoglucanase on Western blots, thus confirming its identity as E1 CAT.

Detailed Description Text (42):

Calorimetric studies of the denaturation of the full size E1 enzyme and the proteolytically cleaved E1 CAT were carried at pH 5.0 in 50 mM sodium acetate, using a Microcal MC-2 differential scanning microcalorimeter over a temperature

range of 25.degree.-95.degree. C. and using a scan rate of 20.degree. C./h. For the examples shown in FIG. 2, the protein concentrations were 0.24 mg/mL for the native E1 enzyme and 0.14 mg/mL for E1 CAT.

Detailed Description Paragraph Table (1):

SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 12 (2)
 INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 30 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (ix) FEATURE: (A) NAME/KEY: E1-f primer (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1: CTCGAGAAAAGAGCGGGCGGCGGCTATTGG30 (2) INFORMATION FOR SEQ ID NO: 2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: Protein (ix) FEATURE: (A) NAME/KEY: E1-f primer (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2: LeuGluLysArgAlaGlyGlyGlyTyrTrp 510 (2) INFORMATION FOR SEQ ID NO: 3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 base pairs (B) TYPE: nucleic Acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (ix) FEATURE: (A) NAME/KEY: E1r (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3: CCTAGGTAACTTGCTGCGCAGGC24 (2) INFORMATION FOR SEQ ID NO: 4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 5 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: Protein (ix) FEATURE: (A) NAME/KEY: E1r (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4: SerAlaAlaCysAla (2) INFORMATION FOR SEQ ID NO: 5: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic Acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5: ATTTTCGATCCTGTCTAATGATCTGCATCGCCTAGC36 (2) INFORMATION FOR SEQ ID NO: 6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: Protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6: SerSerIlePheAspProValGlyAlaSerAlaSerProSerSerGln 51015 (2) INFORMATION FOR SEQ ID NO: 7: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 48 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: TCGTCGATTTTCGATCCTGTGCGCGCTCTGCATCGCCTAGCAGTCAA48 (2) INFORMATION FOR SEQ ID NO: 8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 36 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (ix) FEATURE: (A) NAME/KEY: mutagenic oligo (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: ATTTTCGATCCTGTCTAATGATCTGCATCGCCTAGC36 (2) INFORMATION FOR SEQ ID NO: 9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 48 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (ix) FEATURE: (A) NAME/KEY: mutated DNA (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9: TCGTCGATTTTCGATCCTGTCTAATGATCTGCATCGCCTAGCAGTCAA48 (2) INFORMATION FOR SEQ ID NO: 10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: Protein (ix) FEATURE: (A) NAME/KEY: Mutated amino acid (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: SerSerIlePheAspProValXaaXaaSerAlaSerProSerSerGln 51015 (2) INFORMATION FOR SEQ ID NO: 11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 358 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: Protein (ix) FEATURE: (A) NAME/KEY: E1-CAT (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11: AlaGlyGlyGlyTyrTrpHisThrSerGlyArgGluIleLeuAspAla 151015
 AsnAsnValProValArgIleAlaGlyIleAsnTrpPheGlyPheGlu 202530
 ThrCysAsnTyrValValHisGlyLeuTrpSerArgAspTyrArgSer 354045
 MetLeuAspGlnIleLysSerLeuGlyTyrAsnThrIleArgLeuPro 505560
 TyrSerAspAspIleLeuLysProGlyThrMetProAsnSerIleAsn 65707580
 PheTyrGlnMetAsnGlnAspLeuGlnGlyLeuThrSerLeuGlnVal 859095
 MetAspLysIleValAlaTyrAlaGlyGlnIleGlyLeuArgIleIle 100105110
 LeuAspArgHisArgProAspCysSerGlyGlnSerAlaLeuTrpTyr 115120125
 ThrSerSerValSerGluAlaThrTrpIleSerAspLeuGlnAlaLeu 130135140
 AlaGlnArgTyrLysGlyAsnProThrValValGlyPheAspLeuHis 145150155160
 AsnGluProHisAspProAlaCysTrpGlyCysGlyAspProSerIle 165170175
 AspTrpArgLeuAlaAlaGluAlaGlyAsnAlaValLeuSerVal 180185190
 AsnProAsnLeuLeuIlePheValGluGlyValGlnSerTyrAsnGly 195200205

AspSerTyrTrpTrpGlyGlyAsnLeuGlnGlyAlaGlyGlnTyrPro 210215220
ValValLeuAsnValProAsnArgLeuValTyrSerAlaHisAspTyr 225230235240
AlaThrSerValTyrProGlnThrTrpPheSerAspProThrPhePro 245250255
AsnAsnMetProGlyIleTrpAsnLysAsnTrpGlyTyrLeuPheAsn 260265270
GlnAsnIleAlaProValTrpLeuGlyGluPheGlyThrThrLeuGln 275280285
SerThrThrAspGlnThrTrpLeuLysThrLeuValGlnTyrLeuArg 290295300
ProThrAlaGlnTyrGlyAlaAspSerPheGlnTrpThrPheTrpSer 305310315320
TrpAsnProAspSerGlyAspThrGlyGlyIleLeuLysAspAspTrp 325330335
GlnThrValAspThrValLysAspGlyTyrLeuAlaProIleLysSer 340345350 SerIlePheAspProVal
355358 (2) INFORMATION FOR SEQ ID NO: 12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH:
2293 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY:
linear (ii) MOLECULE TYPE: DNA (ix) FEATURE: (A) NAME/KEY: E1-CAT (xi) SEQUENCE
DESCRIPTION: SEQ ID NO: 12:
GGATCCACGTTGTACAAGGTCACCTGTCCGTCGTTCTGGTAGAGCGGCGGGATGGTCACC60
CGCACGATCTCTCCTTTGTTGATGTGCGACGGTCACGTGGTTACGGTTTGCCTCGGCCGCG120
ATTTTCGCGCTCGGGCTTGCTCCGGCTGTCCGGTTCGGTTTGGCGTGGTGTGCGGAGCAC180
GCCGAGGCGATCCCAATGAGGGCAAGGGCAAGAGCGGAGCCGATGGCACGTCCGGTGGCC240
GATGGGGTACGCCGATGGGGCGTGGCGTCCCCGCCGCGGACAGAACC GGATGCGGAATAG300
GTCACGGTGCACATGTTGCCGTACCGCGGACCCGGATGACAAGGGTGGGTGCGCGGGTC360
GCCTGTGAGCTGCCGGCTGGCGTCTGGATCATGGGAACGATCCCACCATTCCCCGCAATC420
GACGCGATCGGGAGCAGGGCGGCGAGCCGACCGTGTGGTTCGAGCCGGACGATTGCGC480
CATACGGTGTCTGCAATGCCAGCGCCATGTTGTCAATCCGCCAAATGCAGCAATGCACAC540
ATGGACAGGGATTGTGACTCTGAGTAATGATTGGATTGCCTTCTTGCCGCCTACGCGTTA600
CGCAGAGTAGGCGACTGTATGCGGTAGGTTGGCGCTCCAGCCGTGGGCTGGACATGCCTG660
CTGCGAACTCTTGACACGTCTGGTTGAACGCGCAATACTCCCAACACCGATGGGATCGTT720
CCCATAAGTTTCCGTCTCACAACAGAATCGGTGCGCCCTCATGATCAACGTGAAAGGAGT780
ACGGGGGAGAAACAGACGGGGGAGAAACCAACGGGGGATTGGCGGTGCCGCGCGCATTGCG840
GCGAGTGCCTGGCTCGCGGGTGTGCTGCGGGTTCGGCGTCTGTCGTCGCGGTGCTGGCATT900
GGTTGCCGCACTCGCCAACCTAGCCGTGCCGCGGCGGCTCGCGCCGCGGGCGGCGGCTA960
TTGGCACACGAGCGGCCGGGAGATCCTGGACGCGAACAACGTGCCGGTACGGATCGCCGG1020
CATCAACTGGTTTGGGTTGCAAACTGCAATTACGTCTGTCACGGTCTCTGGTCACGCGA1080
CTACCGCAGCATGCTCGACCAGATAAAGTCGCTCGGCTACAACACAATCCGGCTGCCGTA1140
CTCTGACGACATTCTCAAGCCGGGCACCATGCCGAACAGCATCAATTTTTTACCAGATGAA1200
TCAGGACCTGCAGGGTCTGACGTCCTTGACAGGTCTGACAAAATCGTCGCGTACGCCGG1260
TCAGATCGGCCTGCGCATATTCTTGACCGCCACCGACCGGATTGCAGCGGGCAGTCGGC1320
GCTGTGGTACACGAGCAGCGTCTCGGAGGCTACGTGGATTTCGACCTGCAAGCGCTGGC1380
GCAGCGCTACAAGGGAAACCCGACGGTCTGTCGGCTTTGACTTGACAAACGAGCCGCATGA1440
CCCGCCCTGCTGGGGCTGCGGCGATCCGAGCATCGACTGGCGATTGGCCGCCGAGCGGGC1500
CGGAAACGCCGTGCTCTCGGTGAATCCGAACCTGCTCATTTCGTGCAAGGTGTGCAGAG1560
CTACAGCGGAGACTCCTACTGGTGGGGCGGCAACCTGCAAGGAGCCGGCCAGTACCCGGT1620
CGTGCTGAACGTGCCGAACCGCTGGTGTACTCGGCGCAGCACTACGCGACGAGCGTCTA1680
CCCGCAGACGTGGTTTCAGCGATCCGACCTTCCCCAACACATGCCCGGCATCTGGAACAA1740
GAACTGGGGATACCTCTTCAATCAGAACATTGCACCGGTATGGCTGGGCGAATTTCGGTAC1800
GACACTGCAATCCACGACCGACAGACGTGGCTGAAGACGCTCGTCCAGTACCTACGGCC1860
GACCGCGCAATACGGTGCGGACAGCTTCCAGTGGACCTTCTGGTCTTGAACCCCGATTTC1920
CGGCGACACAGGAGGAATTCTCAAGGATGACTGGCAGACGGTCGACACAGTAAAAGACGG1980
CTATCTCGCGCCGATCAAGTCGTGATTTTCGATCCTGTCTAATGATCTGCATCGCCTAG2040
CAGTCAACCGTCCCCGTGGTGTGCGCGTCTCCGTGCGCGAGCCCGTGGCGAGTCGGAC2100
GCCGACGCTACTCCGACGCGGACAGCCAGCCGACGCCAACGCTGACCCCTACTGCTAC2160
GCCACGCCCACGGCAAGCCCCGACGCCGTCAÇCGACGGCAGCCTCCGGAGCCCCGCTGCAC2220
CGCGAGTTACCAGGTCAACAGCGATTGGGGCAATGGCTTCACGGTAACGGTGGCCGTGAC2280 AAATTCGGATCC2293

CLAIMS:

9. The DNA according to claim 7 wherein the DNA encodes a protein having an endoglucanase activity.

13. The DNA according to claim 2 wherein the DNA encodes a protein having an endoglucanase activity.

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